## Mutations and Disease

Mutations in the HFE gene are associated with the hemochromatosis disease. A laboratory working on the hemochromatosis disease wants to elucidate the biochemical and structural basis for the function of the mutant protein.

### Outline:

In this exercise, we have the following goals:
1. Determine what is known about the HFE gene and protein (using Entrez Gene).
2. Determine identified SNPs and their locations in the HFE gene (using dbSNP).
3. Learn more about hemochromatosis and its genetic testing (using OMIM and Gene Tests)
4. Elucidate the biochemical and structural basis for the function of the wild type and mutant proteins, if possible.

## Problem 1

Mutations in the HFE gene are associated with the hemochromatosis disease. A laboratory working on the hemochromatosis disease wants to elucidate the biochemical and structural basis for the function of the mutant protein.

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3. Learning more about the hemochromatosis disease and its genetic testing (using OMIM and Gene Tests)
4. Elucidating the biochemical and structural basis for the function of the wild type and the mutant protein, if possible (using CDD).

### *Step 1. Determining what is known about the HFE gene and protein (using Entrez Gene):*

Search for 'HFE" in Entrez Gene. One entry is for the human HFE gene. Retrieve the entry by clicking on the HFE link.

What is the location and orientation of the HFE gene on the human genome? List the genes adjacent to it. How many alternatively spliced products have been annotated for the HFE gene when the RefSeq mRNA entries were reviewed? Which is the longest splice variant? List some of the HFE gene aliases. What are the phenotypes associated with the mutations in the HFE gene? What is the name and function of the protein encoded by the HFE gene?

## *Step 2. Determining identified SNPs and their locations in the HFE gene:*

From the Links menu on the top right hand side of the page, click on the "SNP: GeneView" to access a list of the known SNPs (reported in dbSNP). By default, the SNPs in the coding region of a gene are reported. Currently, how many missense (non-synonymous) SNPs are placed on the longest hemochromatosis transcript variant, NM_000410? Select the “Include Clinically Associated” SNPs. How many of these have links to OMIM (Clinically Associated)? We will concentrate on the cys282tyr mutant in the following analysis.

## *Step 3. Learning more about the hemochromatosis disease and its genetic testing:*

Click on the OMIM link next to the one of the SNPs in the SNP report. What are the clinical features of hemochromatosis? List the 5 types of iron-overload disorders labeled hemochromatosis. Which of these is associated with mutations in the HFE gene? How many allelic variants of the HFE gene have been reported? What is the phenotype associated with the Cys282Tyr mutant?

Click on the Gene Tests link at top of the page. Identify some of the laboratories performing the clinical testing for hemochromatosis. Now refer to the Reviews section. Mutation analysis is available for which of the HFE alleles? List one explanation for the hemochromatosis phenotype caused by the Cys282Tyr mutant.

## *Step 4. Elucidating the biochemical and structural basis for the function of the wild type and mutant proteins, if possible:*

### A. Visualization of cysteine 282 on the structure of the hemochromatosis protein

Go back to the Entrez Gene report. Click on the protein accession number NP_000401 associated with the longest splice variant NM_000410. Select the GENPEPT link for NP_000401 under the section “Genomic Region, Transcripts and products”. Then select “Related Structure” from the Links menu. The output contains a list of similar proteins with known 3D structures. Set the filters to “All similar MMDB” and sort by “Sequence Identity”. The entry 1A6Z chain A provides the structure of part of human hemochromatosis protein. Click on the first arrow

representing the related structure and then on the “Get 3D-structure data” button. This downloads its 3D structure and the sequence alignment with the query protein. Zoom in to the area of the disulphide bridges (colored in tan) by pressing "z" on the keyboard. Select the cysteine residues forming the disulphide bridges by double clicking on them. Mouse over the corresponding cysteine residues on the query line in the Alignment Viewer and read the amino acid number at the bottom left of the window. One of them is the cysteine at position 282. It is the same cysteine that is mutated to tyrosine causing the hemochromatosis phenotype.

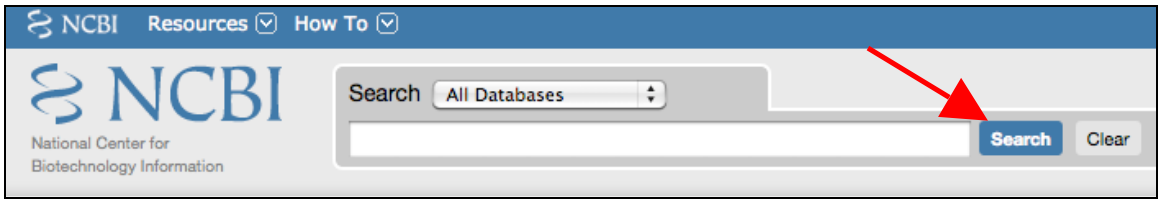
**B. Visualization of hemochromatosis protein and beta-2-microglobulin complex**

Return to the sequence alignment (Related Structures) page and select the link to MMDB (the Molecular Modeling Database). The graphic representation of the structure lists four chains. The PDB record, which can be accessed through the “1A6Z” link on the MMDB page, indicates that chains A and C represent the human hemochromatosis protein, while chains B and D represent human beta-2-microglobulin. Download the structure of the complex by clicking on the structure image on the MMDB page. For easier viewing, remove the helix and strand objects using Style→Edit Global Style -- unclick the boxes next to the Helix objects and Strand objects. To distinguish between the individual chains, select “Molecule” as the Color Scheme for the protein backbone. Click on the “Apply”, then “Done” buttons.

## *You can now easily explain why the C282Y mutant has an altered function.*

**Summary:**
This mini-course describes how to obtain information about the HFE gene, known SNPs in it, and elucidate the biochemical and structural basis for the function of the wild type and Cys282Tyr mutant protein.

Summary: 1. The HFE gene is located on chromosome 6 and has at least 11 alternatively spliced products.
2. Currently, there are 8 non-synonymous SNPs annotated on the protein NP_000401.
3. The Cys282Tyr mutant is associated with the hemochromatosis disease and the site of mutation is used in hemochromatosis genetic testing.
4. The HFE protein functions to regulate iron absorption by regulating the interaction of the transferrin receptor with transferrin whereas the Cys282Tyr mutant fails to regulate this interaction leading to iron overload. The conserved cysteine 282 in the immunoglobulin constant region domain of the HFE protein is involved in formation of a disulphide bridge. Its mutation to tyrosine will alter the folding of the protein.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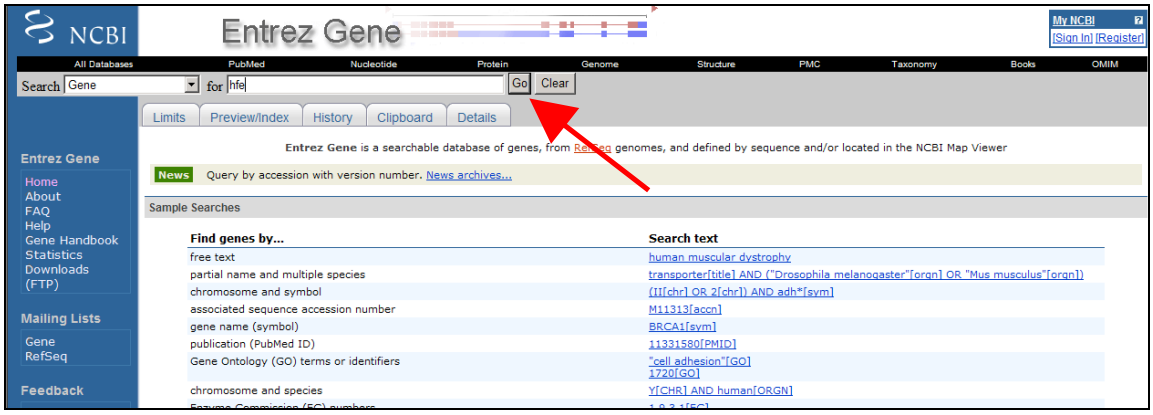
CoreNucleotide: Core subset of nucleotide sequence records
EST: Expressed Sequence Tag records
GSS: Genome Survey Sequence records
Protein: sequence database
Genome: whole genome sequences
Structure: three-dimensional macromolecular structures
Taxonomy: organisms in GenBank
SNP: single nucleotide polymorphism
Gene: gene-centered information
HomoloGene: eukaryotic homology groups
PubChem Compound: unique small molecule chemical structures
PubChem Substance: deposited chemical substance records
Genome Project: genome project information
dbGaP: genotype and phenotype
UniGene: gene-oriented clusters of transcript sequences
CDD: conserved protein domain database
3D Domains: domains from Entrez Structure
UniSTS: markers and mapping data
PopSet: population study data sets
GEO Profiles: expression and molecular abundance profiles
GEO DataSets: experimental sets of GEO data
Cancer Chromosomes: cytogenetic databases
PubChem BioAssay: bioactivity screens of chemical substances
GENSAT: gene expression atlas of mouse central nervous system
Probe: sequence-specific reagents
Protein Clusters: a collection of related protein sequences
Journals: detailed information about the journals indexed in PubMed and other Entrez databases
NLM Catalog: catalog of books, journals, and audiovisuals in the NLM collections
MeSH: detailed information about NLM's controlled vocabulary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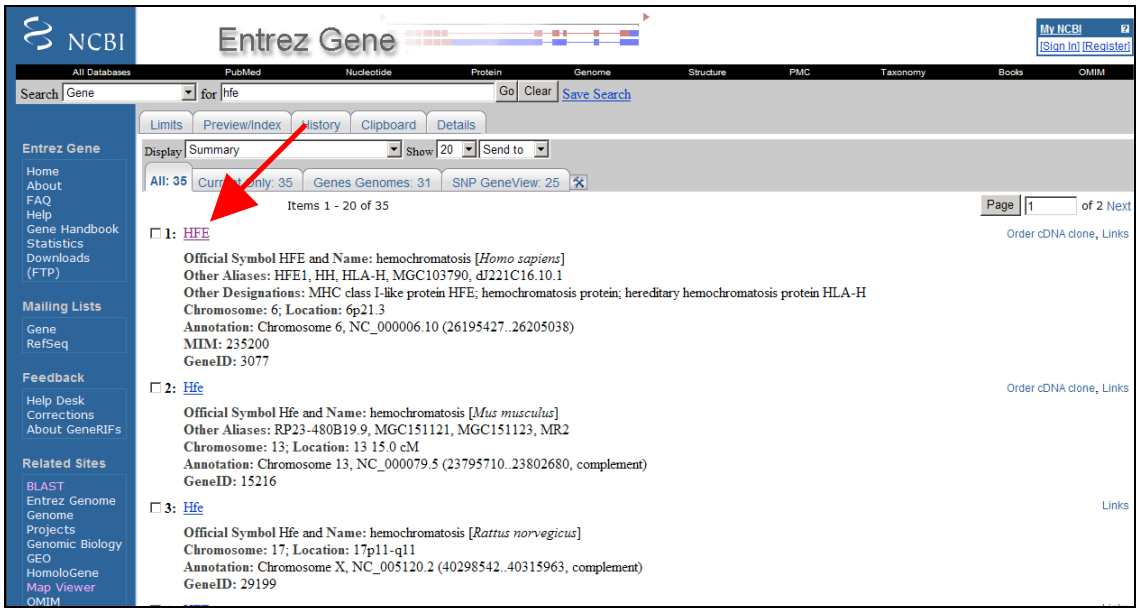
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Related Sites
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Entrez Genome
Genome Projects
Genomic Biology
GEO
HomoloGene
Map Viewer
OMIM
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Items 1 - 20 of 35
Page 1 of 2 Next
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Other Aliases: HFE1, HH, HLA-H, MGC103790, dJ221C16.10.1
Other Designations: MHC class I-like protein HFE; hemochromatosis protein; hereditary hemochromatosis protein HLA-H
Chromosome: 6; Location: 6p21.3
Annotation: Chromosome 6, NC_000006.10 (26195427..26205038)
MIM: 235200
GeneID: 3077
2: Hfe
Order cDNA clone, Links
Official Symbol Hfe and Name: hemochromatosis [Mus musculus]
Other Aliases: RP23-480B19.9, MGC151121, MGC151123, MR2
Chromosome: 13; Location: 13 15.0 cM
Annotation: Chromosome 13, NC_000079.5 (23795710..23802680, complement)
GeneID: 15216
3: Hfe
Links
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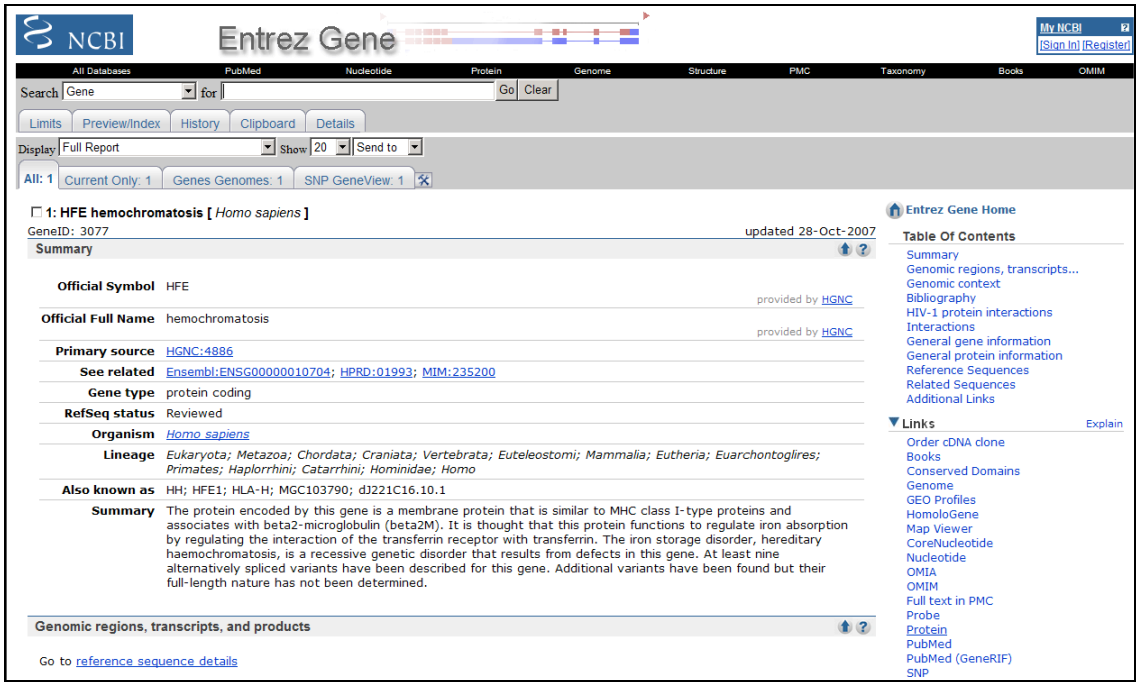
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Display Full Report Show 20 Send to
All: 1 Current Only: 1 Genes Genomes: 1 SNP GeneView: 1
1: HFE hemochromatosis [ Homo sapiens ]
GeneID: 3077 updated 28-Oct-2007
Summary
Official Symbol HFE provided by HGNC
Official Full Name hemochromatosis provided by HGNC
Primary source HGNC:4886
See related Ensembl:ENSG00000010704; HPRD:01993; MIM:235200
Gene type protein coding
RefSeq status Reviewed
Organism Homo sapiens
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as HH; HFE1; HLA-H; MGC103790; dJ221C16.10.1
Summary The protein encoded by this gene is a membrane protein that is similar to MHC class I-type proteins and associates with beta2-microglobulin (beta2M). It is thought that this protein functions to regulate iron absorption by regulating the interaction of the transferrin receptor with transferrin. The iron storage disorder, hereditary haemochromatosis, is a recessive genetic disorder that results from defects in this gene. At least nine alternatively spliced variants have been described for this gene. Additional variants have been found but their full-length nature has not been determined.
Genomic regions, transcripts, and products
Go to reference sequence details
Entrez Gene Home
Table Of Contents
Summary
Genomic regions, transcripts...
Genomic context
Bibliography
HIV-1 protein interactions
Interactions
General gene information
General protein information
Reference Sequences
Related Sequences
Additional Links
Links Explain
Order cDNA clone
Books
Conserved Domains
Genome
GEO Profiles
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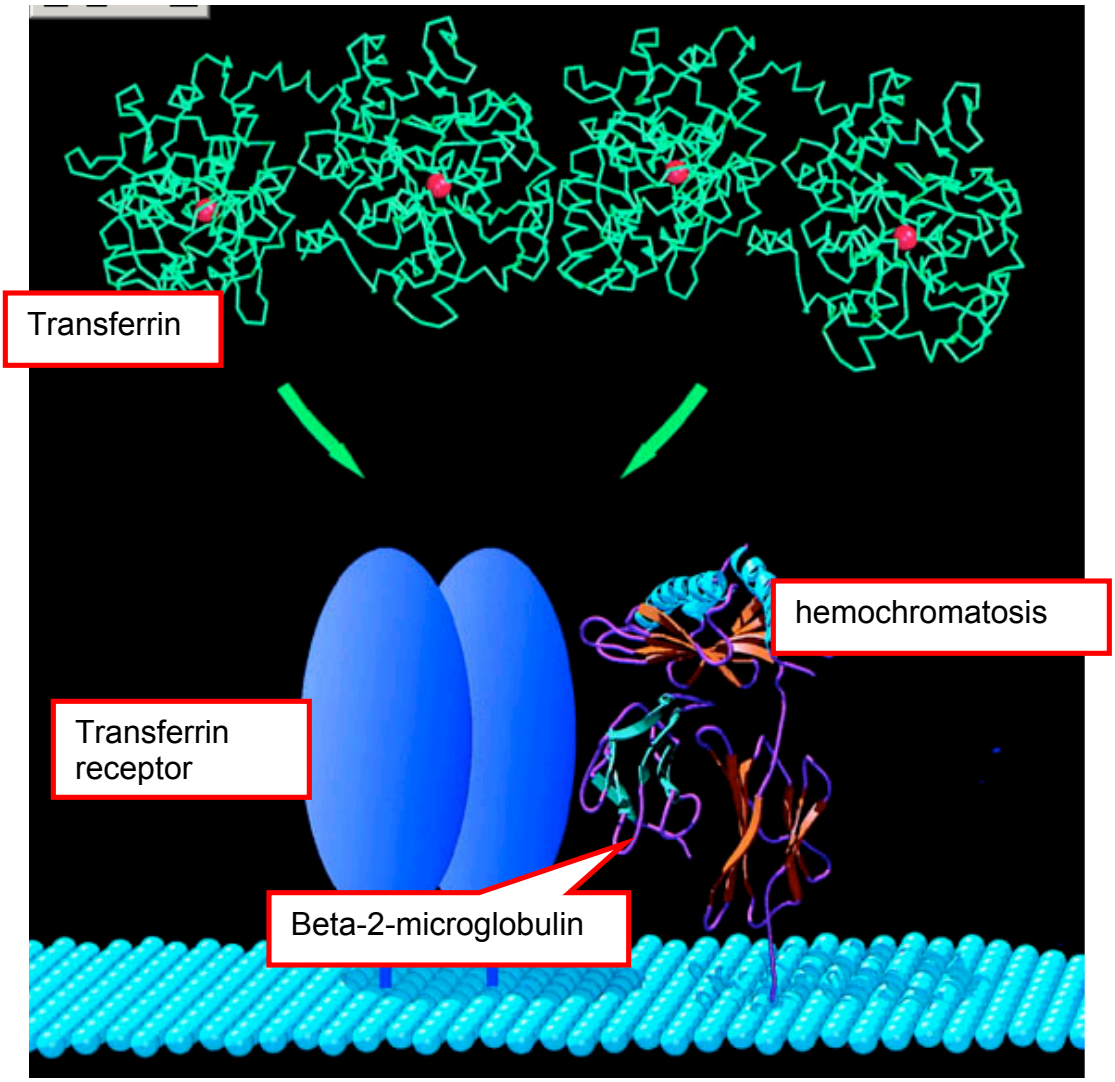
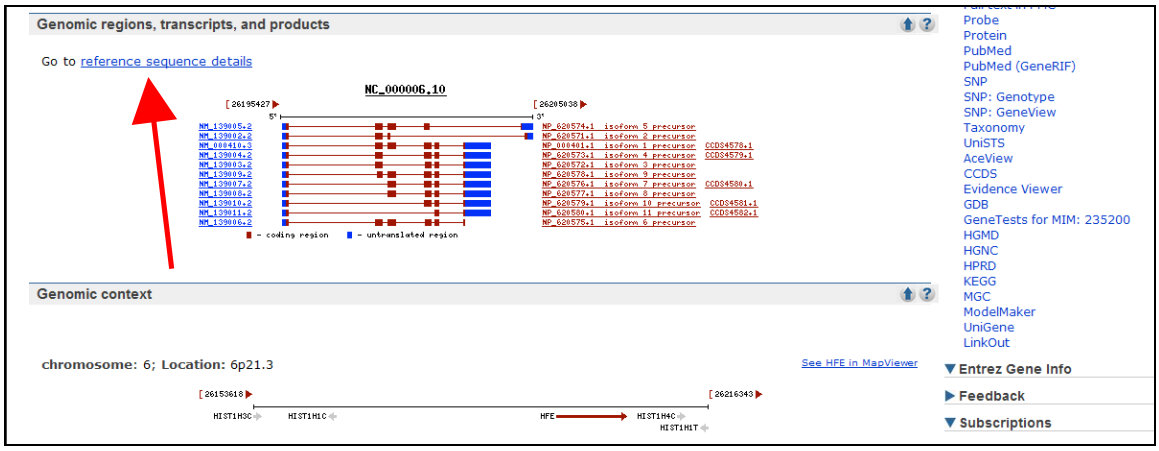
SNP




**Bacon et al. Gastroenterology, 116:193-207, Figure 4**

**The interaction of hemochromatosis protein with beta-2-microglobulin allows cell surface presentation of the complex. Once on cell surface, the hemochromatosis protein regulates iron absorption by regulating the interaction of the transferrin receptor with transferrin.**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NC_000006.10
Genomic context
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See HFE in MapViewer
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HIST1H1C
HFE
HIST1H4C
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General protein information
Names
hemochromatosis protein
MHC class I-like protein HFE
hereditary hemochromatosis protein HLA-H
NCBI Reference Sequences (RefSeq)
Genomic
1. NG_001335.1 Reference
Range 71162..80773
Download GenBank FASTA
mRNA and Protein(s)
1. NM_000410.3→NP_000401.1 hemochromatosis protein isoform 1 precursor
Description Transcript Variant: This variant (1) encodes the longest isoform.
Source sequence(s) AF115265,AJ249337,U91328
Consensus CDS CCDS4578.1
Conserved Domains (2) summary
cd00098 Location:223-298 Blast Score:169
IGc; Immunoglobulin domain constant region subfamily; members of the IGc subfamily are components of immunoglobulins, T-cell receptors, CD1 cell surface glycoproteins, secretory glycoproteins A/C, and Major Histocompatibility Complex (MHC) class I/II molecules
pfam00129 Location:27-202 Blast Score:314
MHC_I; Class I Histocompatibility antigen, domains alpha 1 and 2
2. NM_139002.2→NP_620571.1 hemochromatosis protein isoform 2 precursor
Description Transcript Variant: This variant (2) lacks a large 3' region including the 3' CDS and UTR but has an alternate 3' exon, as compared to variant 1. The resulting protein (isoform 2) has a unique carboxy terminus.

PubMed links

**GeneRIFs: Gene References Into Function** What's a GeneRIF?

important to recognise the symptoms of iron overloading at an early stage because hereditary haemochromatosis needs to be treated immediately.

151. The effect of particlete air pollution on cardiac autonomic function was shielded in subjects with at least 1 copy of an HFE variant compared with wild-type subjects.

152. Our data suggest that the HFE gene is not a major disease gene for migraine.

153. analysis of the localisation and functional effects of the HFE and its chaperone protein beta2M

154. Prevalence of epsilon dA was significantly higher in specimens of alcoholic fatty liver and fibrosis patients but not in hepatitis samples. The prevalence in alcohol fibrosis was as high as in the liver from Wilson's disease and hemochromatosis patients.

155. The Ala176Val mutation may have a possible role on the cause of hemochromatosis in Japanese case

156. REVIEW: C282Y mutant gene product failed to associate with 2-microglobulin and significantly reduced cell surface expression of the HFE-2m complex, thereby affecting the interaction with TfR and its interaction with transferrin.

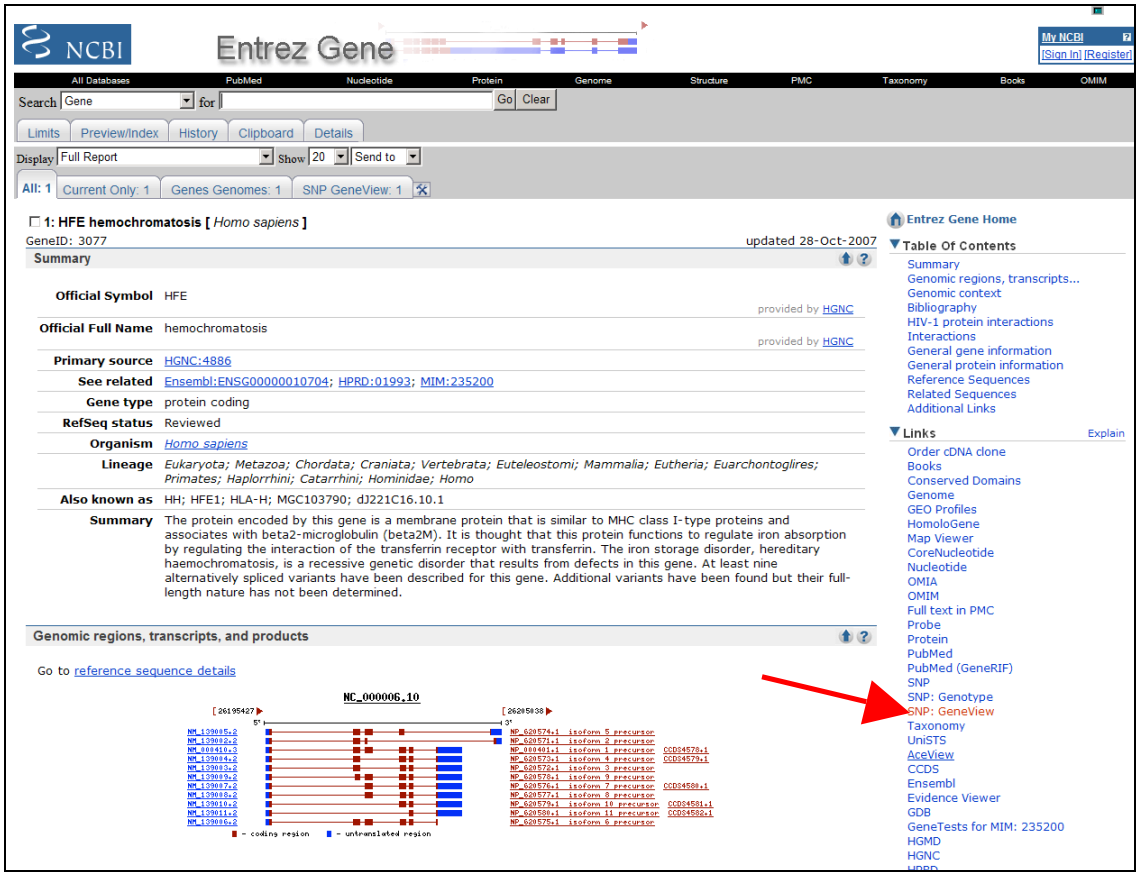
157. 871 healthy unrelated subjects in Poland were collected to assess the relevant frequencies. Each subject was genotyped for the C282Y and H63D mutations using a PCR-based protocol

**Submit:** New GeneRIF Correction

**Interactions**

**Description .....**

| Product | Interactant | Other Gene | Complex | Source | Pubs |
|---|---|---|---|---|---|
| NP_000401.1 | Beta 2 microglobulin | B2M | | HPRD | PubMed |
| NP_000401.1 | Transferrin receptor 2 | TFR2 | | HPRD | PubMed |
| NP_000401.1 | NP_003225.1 | TFRC | | HPRD | PubMed |
| in vitro | | | | | |
| BioGRID:109325 | BioGRID:107044 | B2M | | BioGRID | PubMed |
| in vivo | | | | | |
| BioGRID:109325 | BioGRID:112894 | TFR2 | | BioGRID | PubMed |
| in vitro; in vivo | | | | | |
| BioGRID:109325 | BioGRID:112895 | TFRC | | BioGRID | PubMed |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All: 1 Current Only: 1 Genes Genomes: 1 SNP GeneView: 1
1: HFE hemochromatosis [ Homo sapiens ]
GeneID: 3077
updated 28-Oct-2007
Summary
Official Symbol HFE
provided by HGNC
Official Full Name hemochromatosis
provided by HGNC
Primary source HGNC:4886
See related Ensembl:ENSG00000010704; HPRD:01993; MIM:235200
Gene type protein coding
RefSeq status Reviewed
Organism Homo sapiens
Lineage Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini; Catarrhini; Hominidae; Homo
Also known as HH; HFE1; HLA-H; MGC103790; dJ221C16.10.1
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Genomic regions, transcripts, and products
Go to reference sequence details
NC_000006.10
- coding region - untranslated region
Entrez Gene Home
Table Of Contents
Summary
Genomic regions, transcripts...
Genomic context
Bibliography
HIV-1 protein interactions
Interactions
General gene information
General protein information
Reference Sequences
Related Sequences
Additional Links
Links Explain
Order cDNA clone
Books
Conserved Domains
Genome
GEO Profiles
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CoreNucleotide
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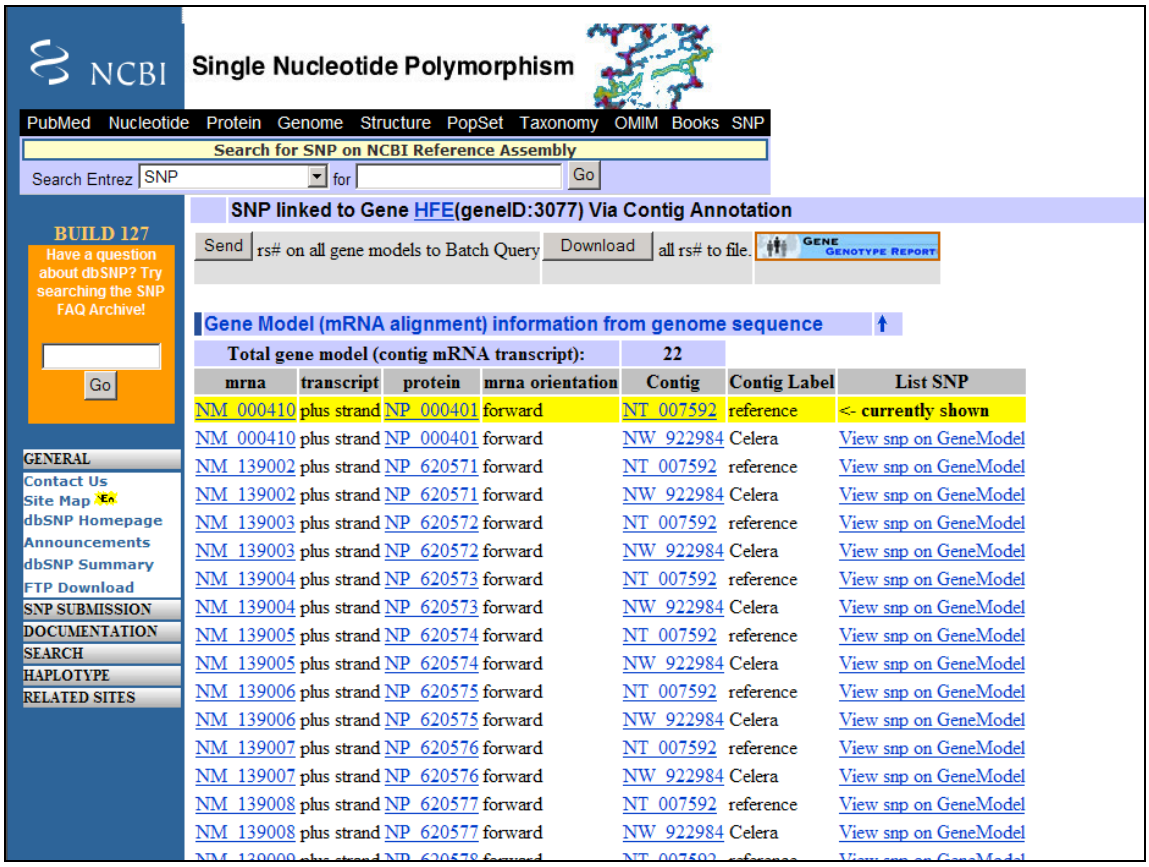


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Nomenclature
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History
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View List
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Gene map
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HEMOCHROMATOSIS; HFE
GeneTests, Links
Alternative titles; symbols
HLAH
HEMOCHROMATOSIS, HEREDITARY; HH
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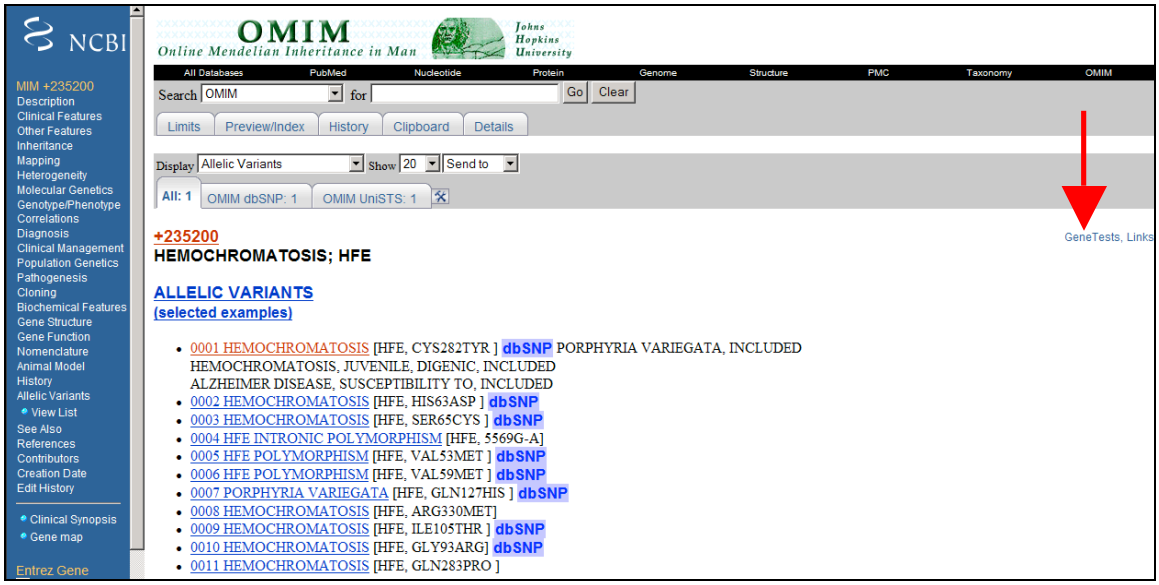
Gene map locus 6p21.3
TEXT
DESCRIPTION
The clinical features of hemochromatosis include cirrhosis of the liver, diabetes, hypermelanotic pigmentation of the skin, and heart failure. Primary hepatocellular carcinoma (HCC; 114550), complicating cirrhosis, is responsible for about one-third of deaths in affected homozygotes. Since hemochromatosis is a relatively easily treated disorder if diagnosed, this is a form of preventable cancer.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Gene Function
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HEMOCHROMATOSIS; HFE
GeneTests, Links
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HEMOCHROMATOSIS, JUVENILE, DIGENIC, INCLUDED
ALZHEIMER DISEASE, SUSCEPTIBILITY TO, INCLUDED
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0003 HEMOCHROMATOSIS [HFE, SER65CYS ] dbSNP
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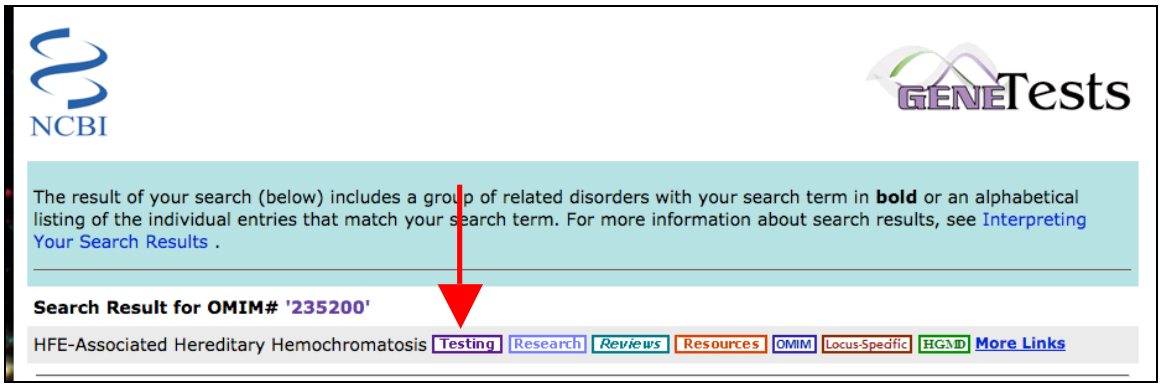
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Locus-Specific
HGMD
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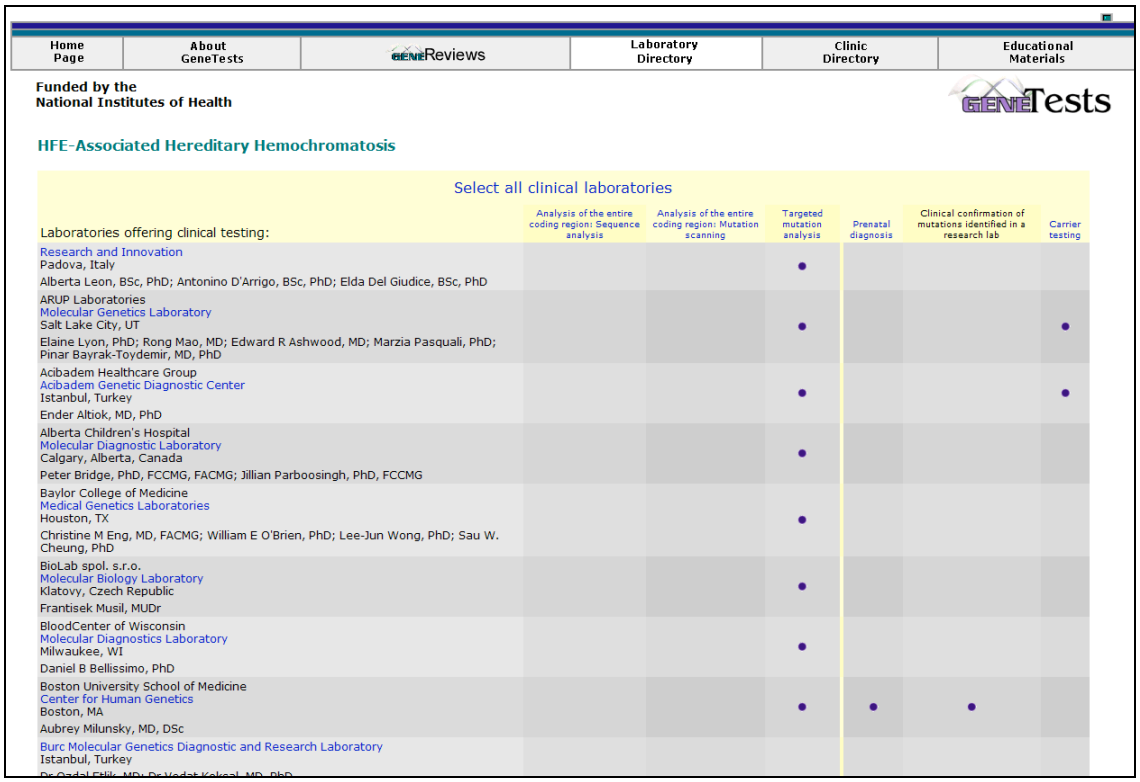
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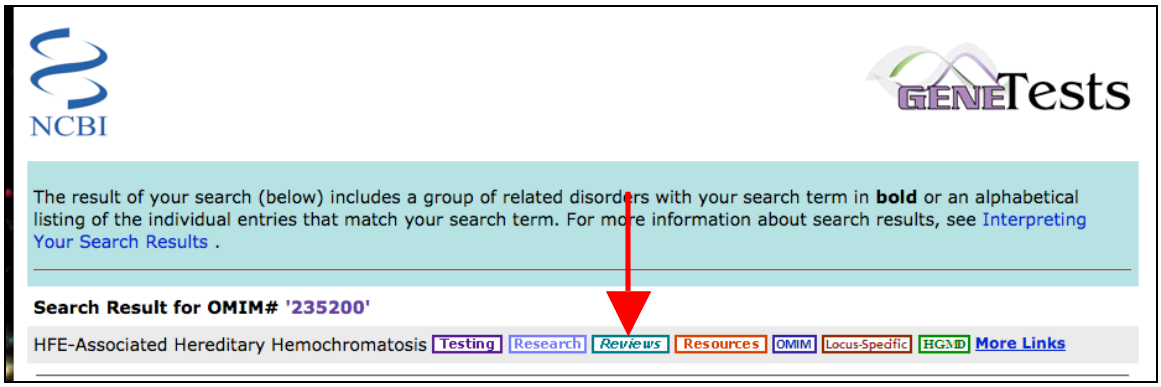
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Reviews
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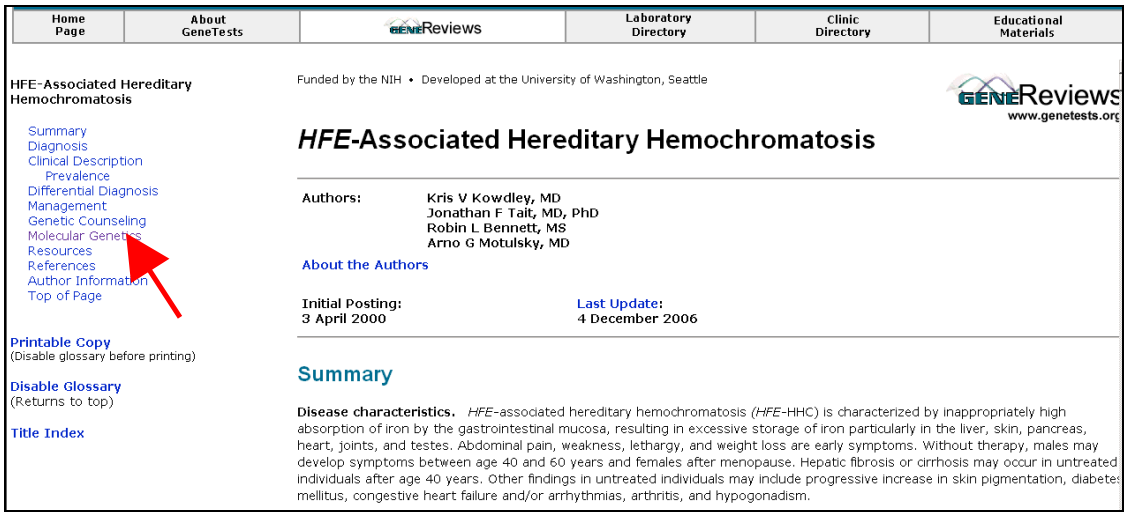
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Summary
Diagnosis
Clinical Description
Prevalence
Differential Diagnosis
Management
Genetic Counseling
Molecular Genetics
Resources
References
Author Information
Top of Page
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GeneReviews
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HFE-Associated Hereditary Hemochromatosis
Authors:
Kris V Kowdley, MD
Jonathan F Tait, MD, PhD
Robin L Bennett, MS
Arno G Motulsky, MD
About the Authors
Initial Posting:
3 April 2000
Last Update:
4 December 2006
Summary
Disease characteristics. HFE-associated hereditary hemochromatosis (HFE-HHC) is characterized by inappropriately high absorption of iron by the gastrointestinal mucosa, resulting in excessive storage of iron particularly in the liver, skin, pancreas, heart, joints, and testes. Abdominal pain, weakness, lethargy, and weight loss are early symptoms. Without therapy, males may develop symptoms between age 40 and 60 years and females after menopause. Hepatic fibrosis or cirrhosis may occur in untreated individuals after age 40 years. Other findings in untreated individuals may include progressive increase in skin pigmentation, diabetes mellitus, congestive heart failure and/or arrhythmias, arthritis, and hypogonadism.

**Table A. HFE-Associated Hereditary Hemochromatosis: Genes and Databases**

| Gene Symbol | Chromosomal Locus | Protein Name | Locus Specific | HGMD |
|---|---|---|---|---|
| *HFE* | 6p21.3 | Hereditary hemochromatosis protein | alsod/HFE genetic mutations | HFE<br>TF |

Data are compiled from the following standard references: gene symbol from HGNC; chromosomal locus, locus name, critical region, complementation group from OMIM; protein name from UniProt. For a description of databases (Locus Specific, HGMD) linked to, click here.

**Table B. OMIM Entries for HFE-Associated Hereditary Hemochromatosis (View All in OMIM)**

| | |
|---|---|
| 235200 | HEMOCHROMATOSIS; HFE |

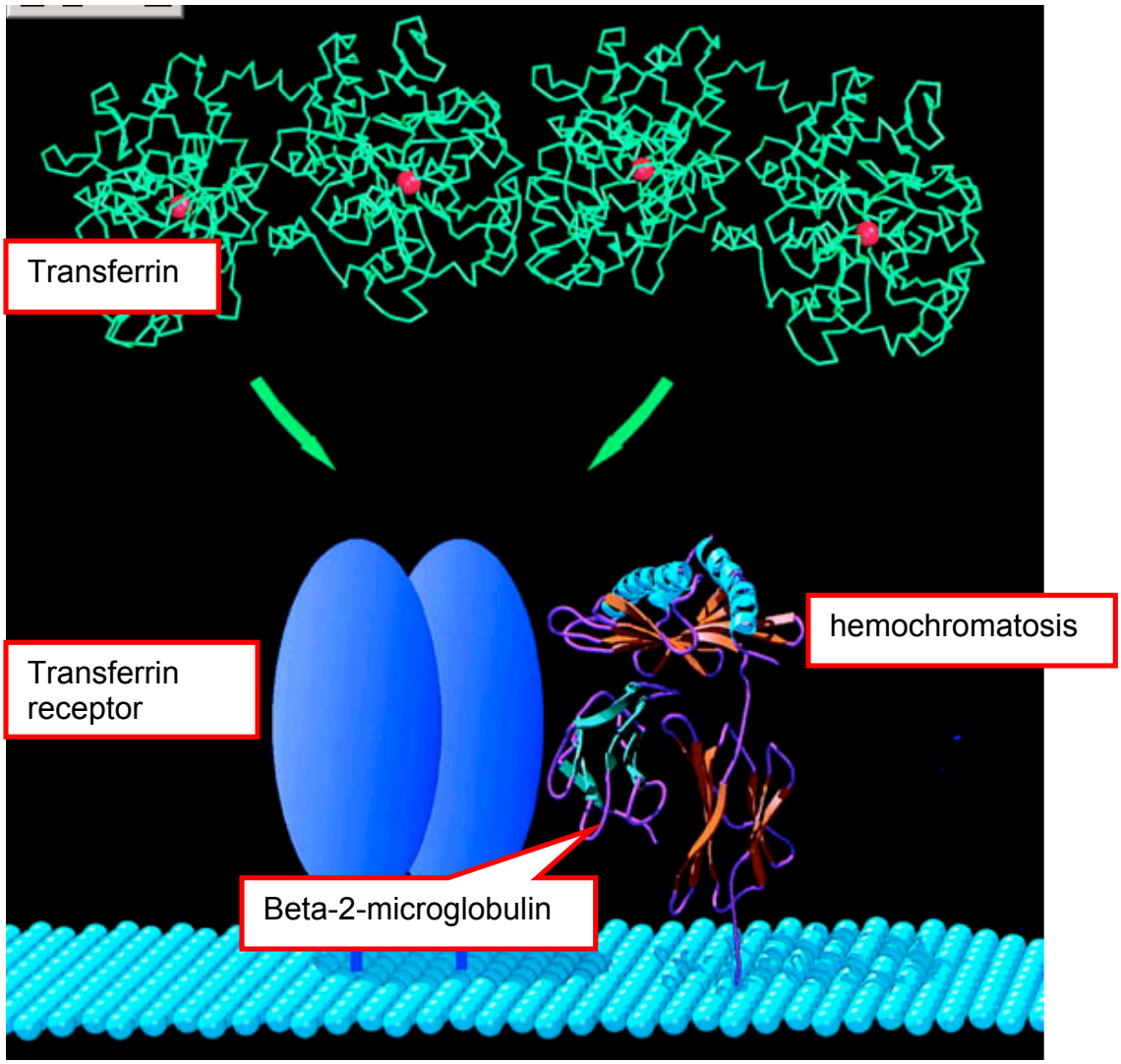
**Normal allelic variants:** The *HFE* gene is about 13 kb in size and contains seven exons [Feder et al 1996, Albig 1998]; *HFE* gives rise to at least eleven alternative transcripts encoding four to seven exons.

**Pathologic allelic variants:** At least 28 distinct mutations have been reported, most being missense or nonsense mutations. Two missense mutations account for the vast majority of disease-causing alleles in the population:

- Cys282Tyr (p.C282Y; nucleotide 845G>A). This missense mutation removes a highly conserved cysteine residue that normally forms an intermolecular disulfide bond with beta-2-microglobulin, and thereby prevents the protein from being expressed on the cell surface.
- His63Asp (p.H63D; nucleotide 187C>G). This missense mutation may alter a pH-dependent intramolecular salt bridge, possibly affecting interaction of the HFE protein with the transferrin receptor.

**Normal gene product:** The largest predicted primary translation product is 348 amino acids, which gives rise to a mature protein of about 321 amino acids after cleavage of the signal sequence. The HFE protein is similar to HLA Class I molecules at the primary [Feder et al 1996] and tertiary structure [Lebron et al 1998] levels. The mature protein is expressed on the cell surface as a heterodimer with beta-2-microglobulin, and this interaction is necessary for normal presentation on the cell surface. The normal HFE protein binds to transferrin receptor 1 on the cell surface and may reduce cellular iron uptake; however, the exact means by which the HFE protein regulates iron uptake is as yet unclear [Fleming et al 2004].

**Abnormal gene product:** The p.C282Y mutation destroys a key cysteine residue that is required for disulfide bonding with beta-2-microglobulin. As a result, the HFE protein does not mature properly and becomes trapped in the endoplasmic reticulum and Golgi apparatus, leading to decreased cell-surface expression. The mechanistic basis for the phenotypic effect of other *HFE* mutations is not clear at present.




**Bacon et al. Gastroenterology, 116:193-207, Figure 4**

**The interaction of hemochromatosis protein with beta-2-microglobulin allows cell surface presentation of the complex. Once on cell surface, the hemochromatosis protein regulates iron absorption by regulating the interaction of the transferrin receptor with transferrin.**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Other Features
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Genotype/Phenotype
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Clinical Management
Population Genetics
Pathogenesis
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Biochemical Features
Gene Structure
Gene Function
Nomenclature
Animal Model
History
Allelic Variants
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See Also
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Contributors
Creation Date
Edit History
Clinical Synopsis
Gene map
+235200
HEMOCHROMATOSIS; HFE
Alternative titles; symbols
HLAH
HEMOCHROMATOSIS, HEREDITARY; HH
HFE GENE, INCLUDED; HFE, INCLUDED
Gene map locus 6p21.3
TEXT
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The clinical features of hemochromatosis include cirrhosis of the liver, diabetes, hypermelanotic pigmentation of the skin, and heart failure. Prir
(HCC; 114550), complicating cirrhosis, is responsible for about one-third of deaths in affected homozygotes. Since hemochromatosis is a relatr
diagnosed, this is a form of preventable cancer.
Links
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HomoloGene
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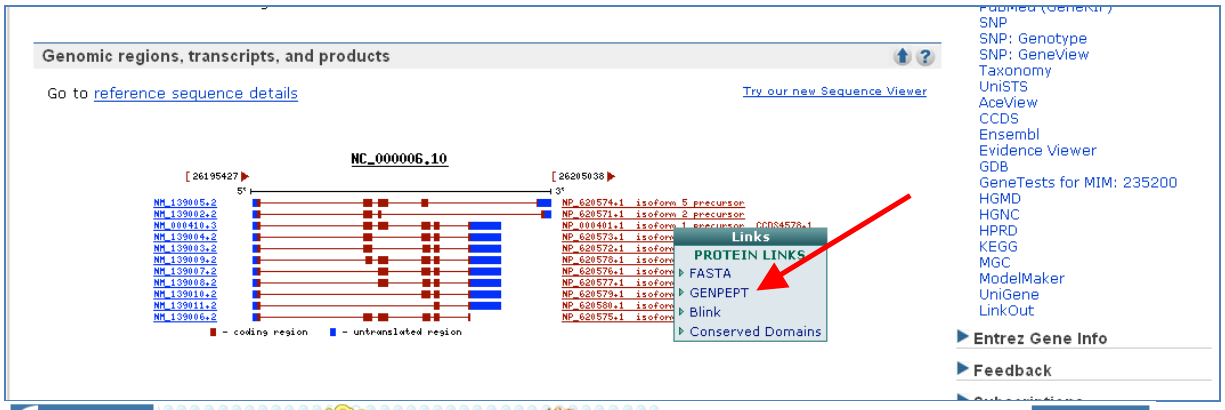
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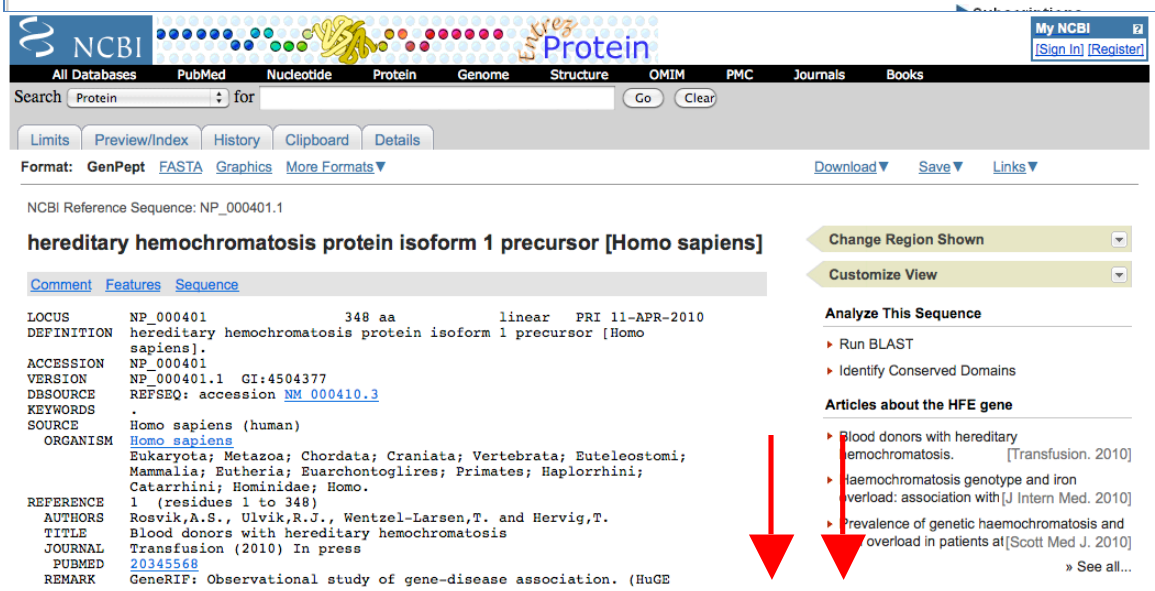
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Format: GenPept FASTA Graphics More Formats
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ORGANISM Homo sapiens
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TITLE Blood donors with hereditary hemochromatosis
JOURNAL Transfusion (2010) In press
PUBMED 20345568
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Blood donors with hereditary hemochromatosis. [Transfusion. 2010]
Haemochromatosis genotype and iron overload: association with [J Intern Med. 2010]
Prevalence of genetic haemochromatosis and overload in patients at [Scott Med J. 2010]
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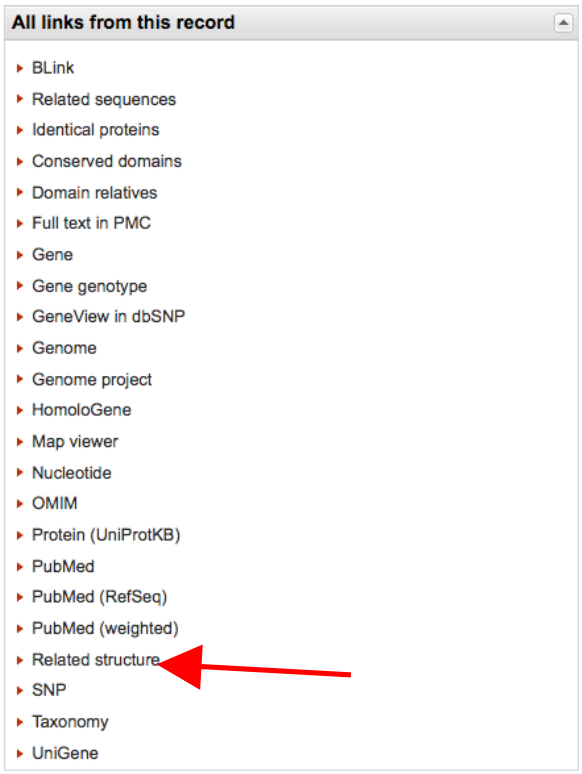
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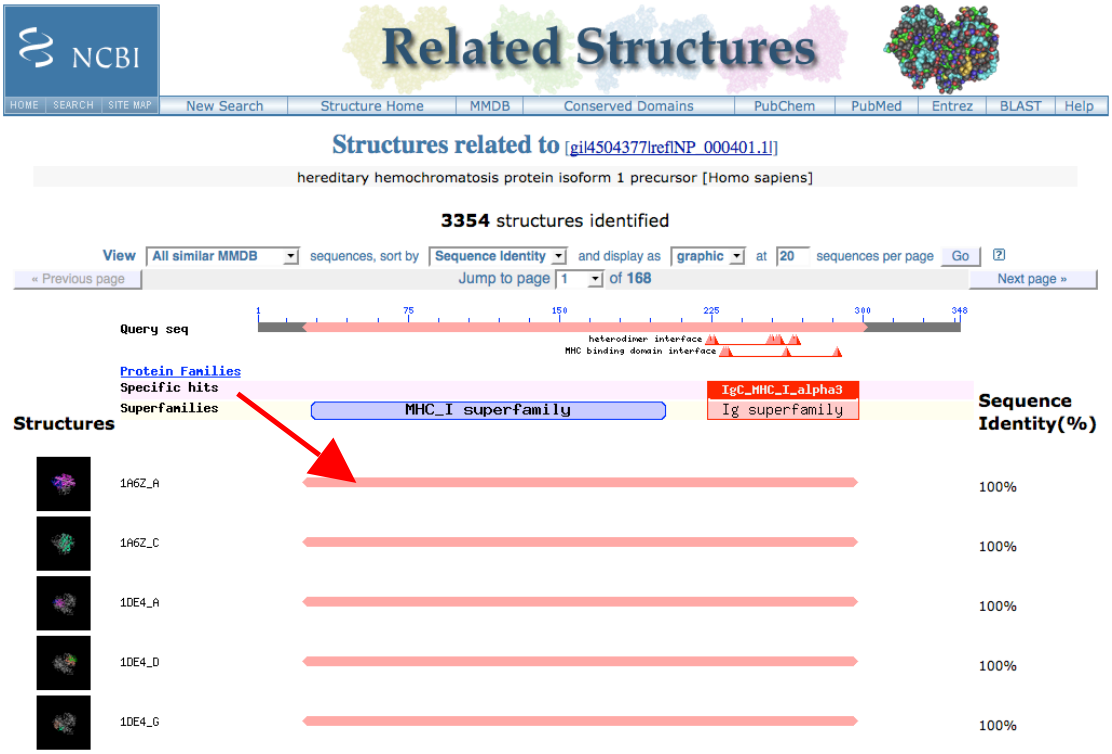
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MHC binding domain interface
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Superfamilies
IgC_MHC_I_alpha3
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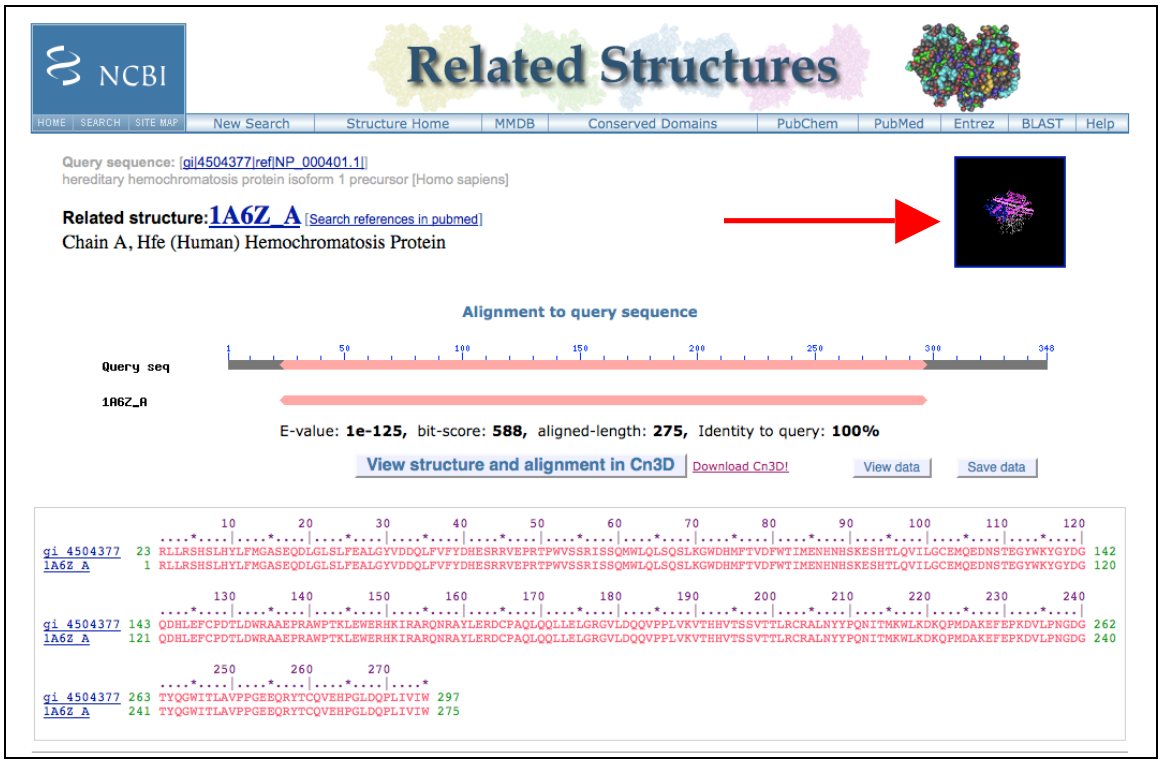
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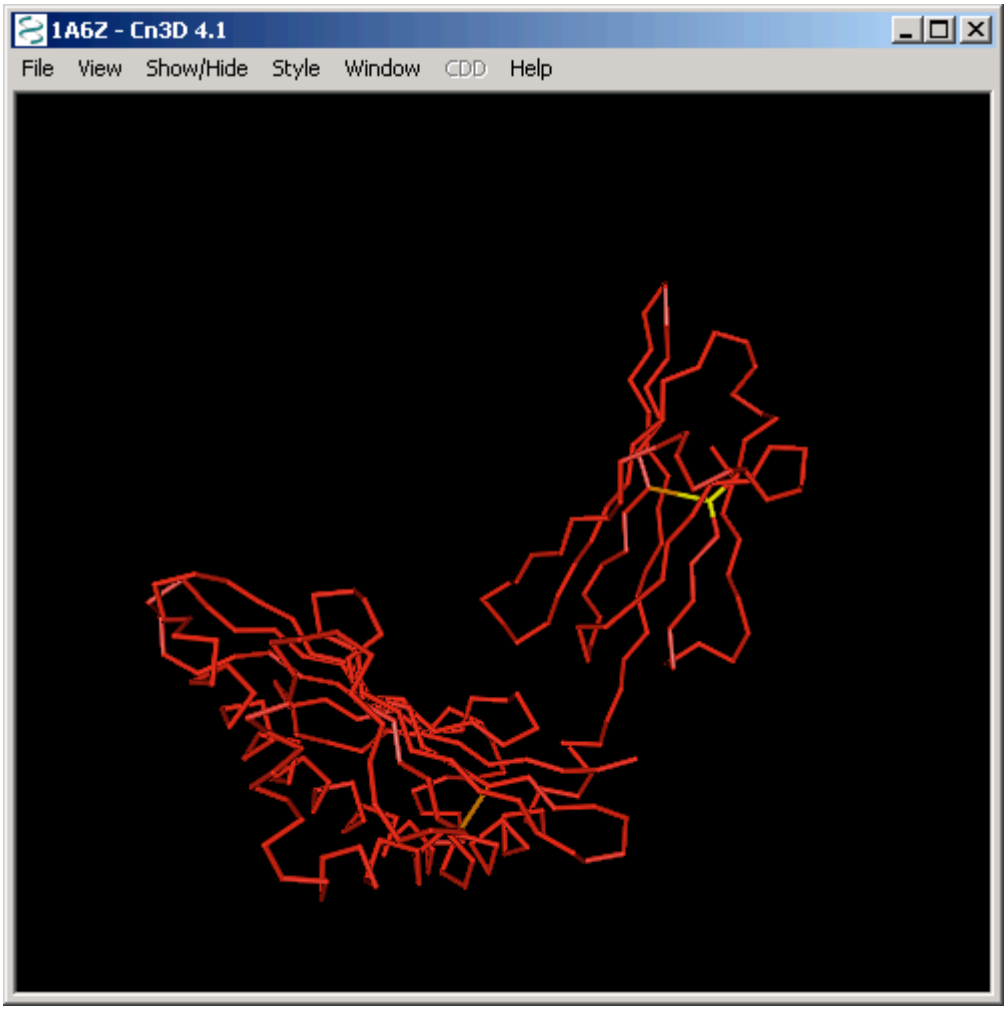
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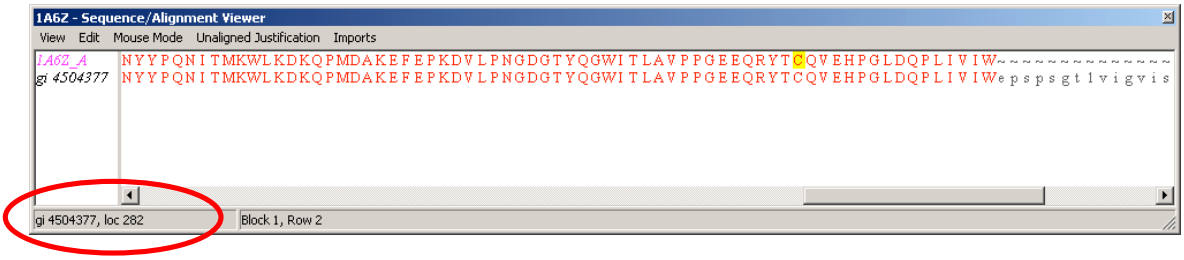
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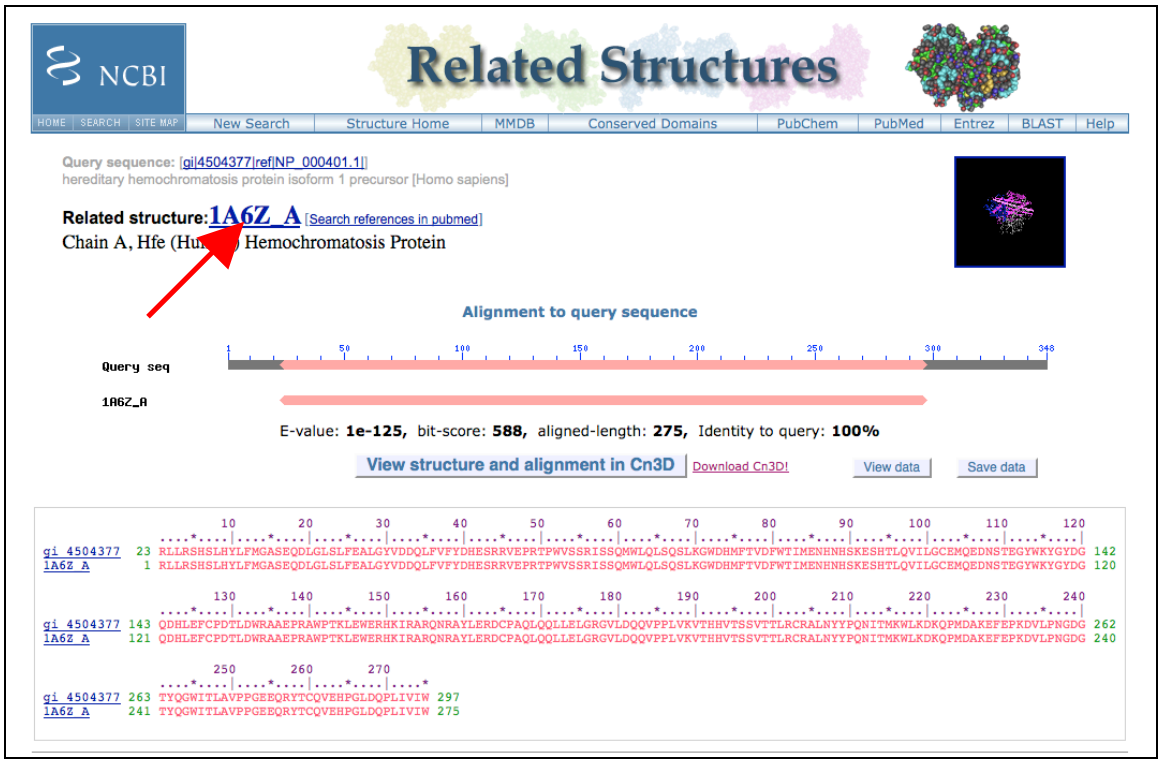
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1A6Z_A
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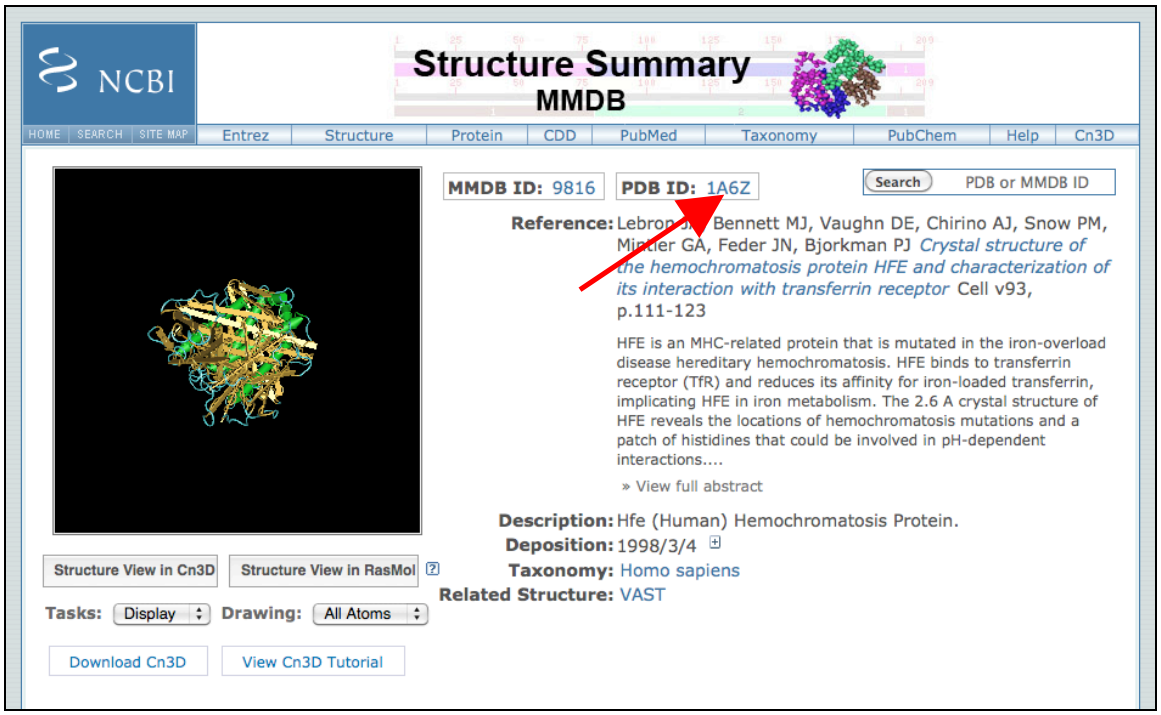
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HFE is an MHC-related protein that is mutated in the iron-overload disease hereditary hemochromatosis. HFE binds to transferrin receptor (TfR) and reduces its affinity for iron-loaded transferrin, implicating HFE in iron metabolism. The 2.6 A crystal structure of HFE reveals the locations of hemochromatosis mutations and a patch of histidines that could be involved in pH-dependent interactions....
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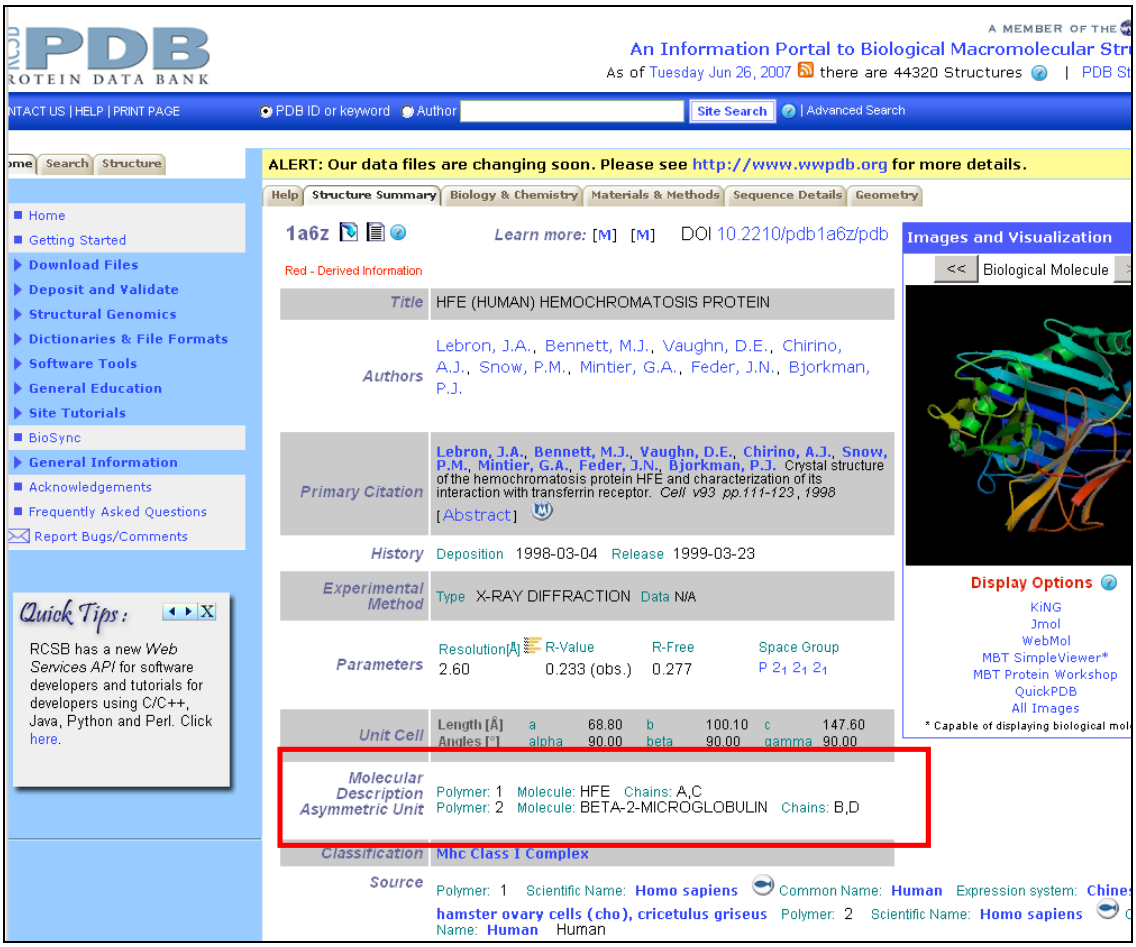
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DOI 10.2210/pdb1a6z/pdb
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Title HFE (HUMAN) HEMOCHROMATOSIS PROTEIN
Authors Lebron, J.A., Bennett, M.J., Vaughn, D.E., Chirino, A.J., Snow, P.M., Mintier, G.A., Feder, J.N., Bjorkman, P.J.
Primary Citation Lebron, J.A., Bennett, M.J., Vaughn, D.E., Chirino, A.J., Snow, P.M., Mintier, G.A., Feder, J.N., Bjorkman, P.J. Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor. Cell v93 pp.111-123, 1998
[Abstract]
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Experimental Method Type X-RAY DIFFRACTION Data N/A
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Classification Mhc Class I Complex
Source Polymer: 1 Scientific Name: Homo sapiens Common Name: Human Expression system: Chine hamster ovary cells (cho), cricetulus griseus Polymer: 2 Scientific Name: Homo sapiens Name: Human Human
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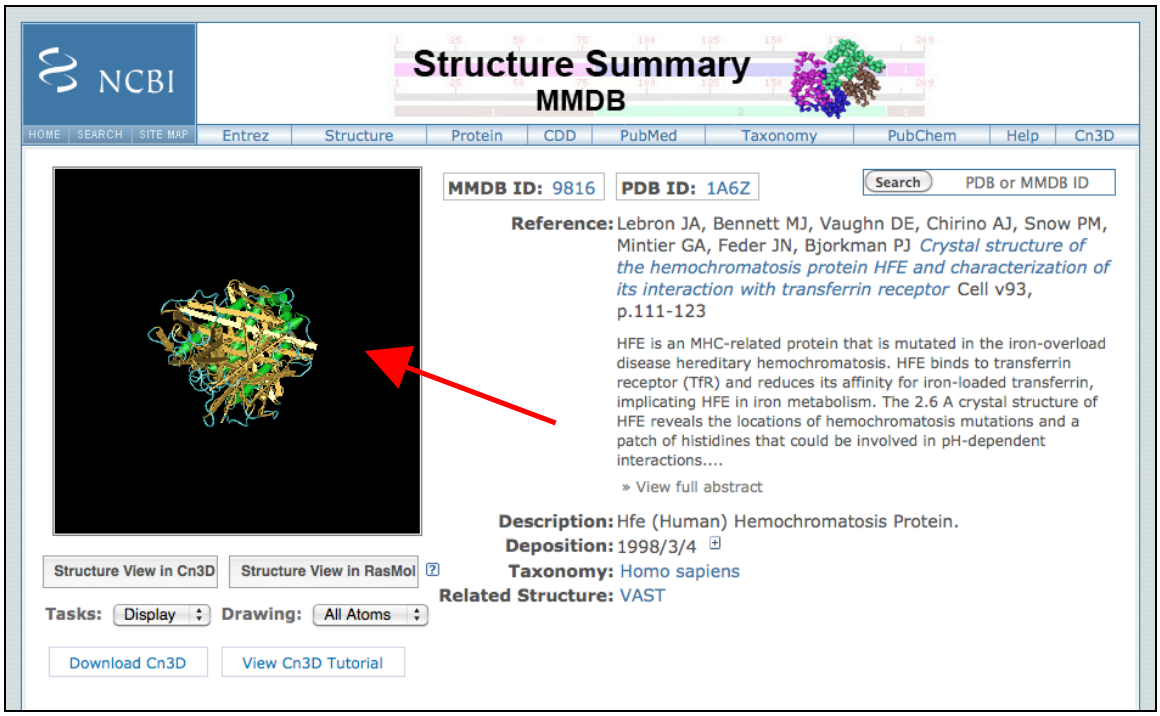
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Reference: Lebron JA, Bennett MJ, Vaughn DE, Chirino AJ, Snow PM, Mintier GA, Feder JN, Bjorkman PJ Crystal structure of the hemochromatosis protein HFE and characterization of its interaction with transferrin receptor Cell v93, p.111-123
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Deposition: 1998/3/4
Taxonomy: Homo sapiens
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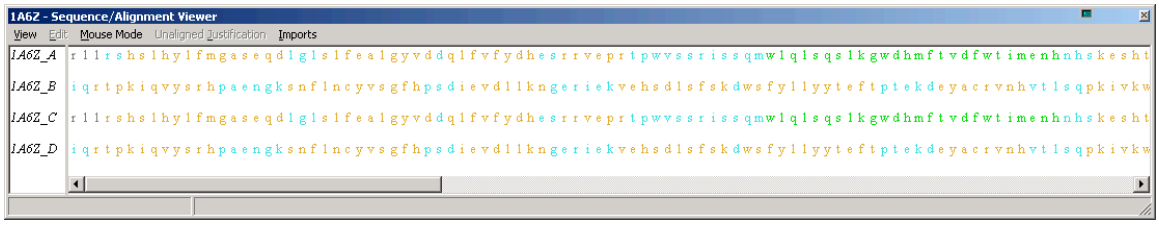
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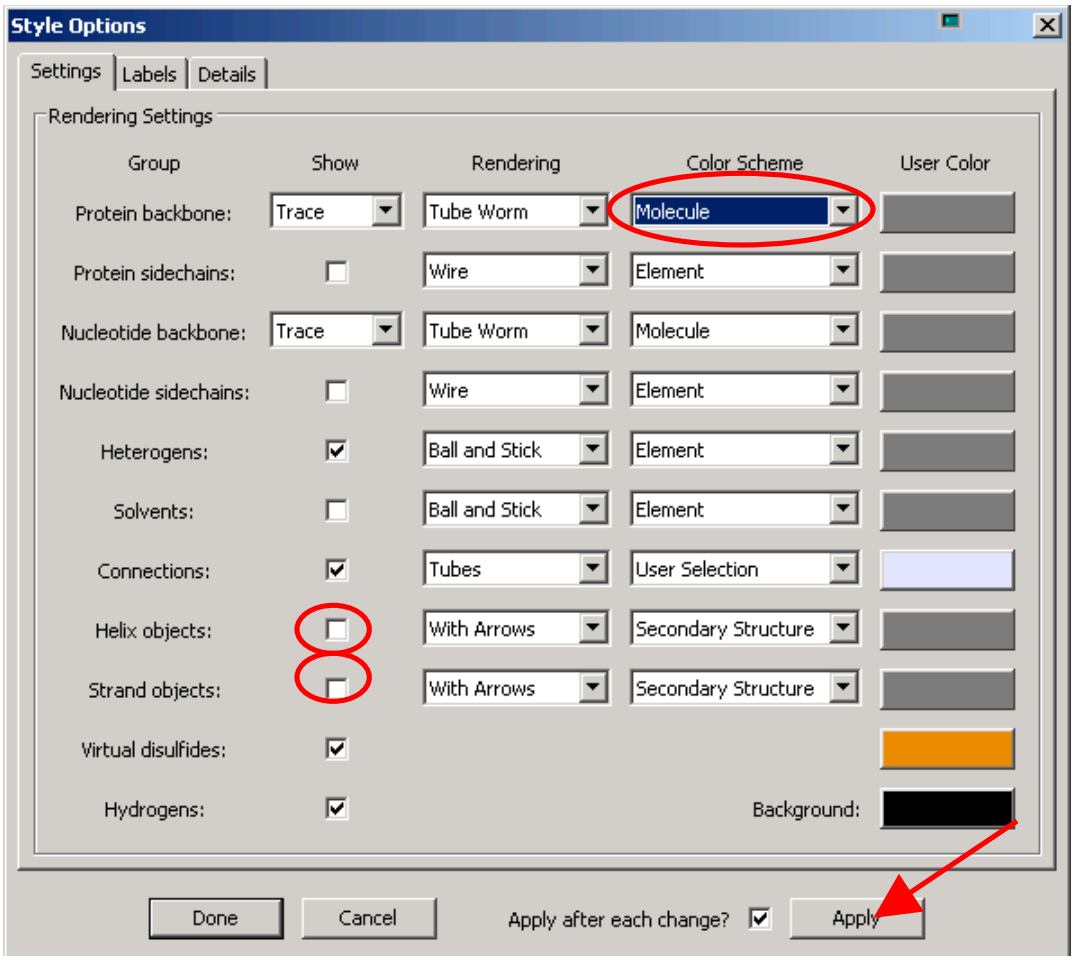
1A6Z_D i q r t p k i q v y s r h p a e n g k s n f l n c y v s g f h p s d i e v d l l k n g e r i e k v e h s d l s f s k d w s f y l l y y t e f t p t e k d e y a c r v n h v t l s q p k i v k w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Element
Nucleotide backbone:
Trace
Tube Worm
Molecule
Nucleotide sidechains:
Wire
Element
Heterogens:
Ball and Stick
Element
Solvents:
Ball and Stick
Element
Connections:
Tubes
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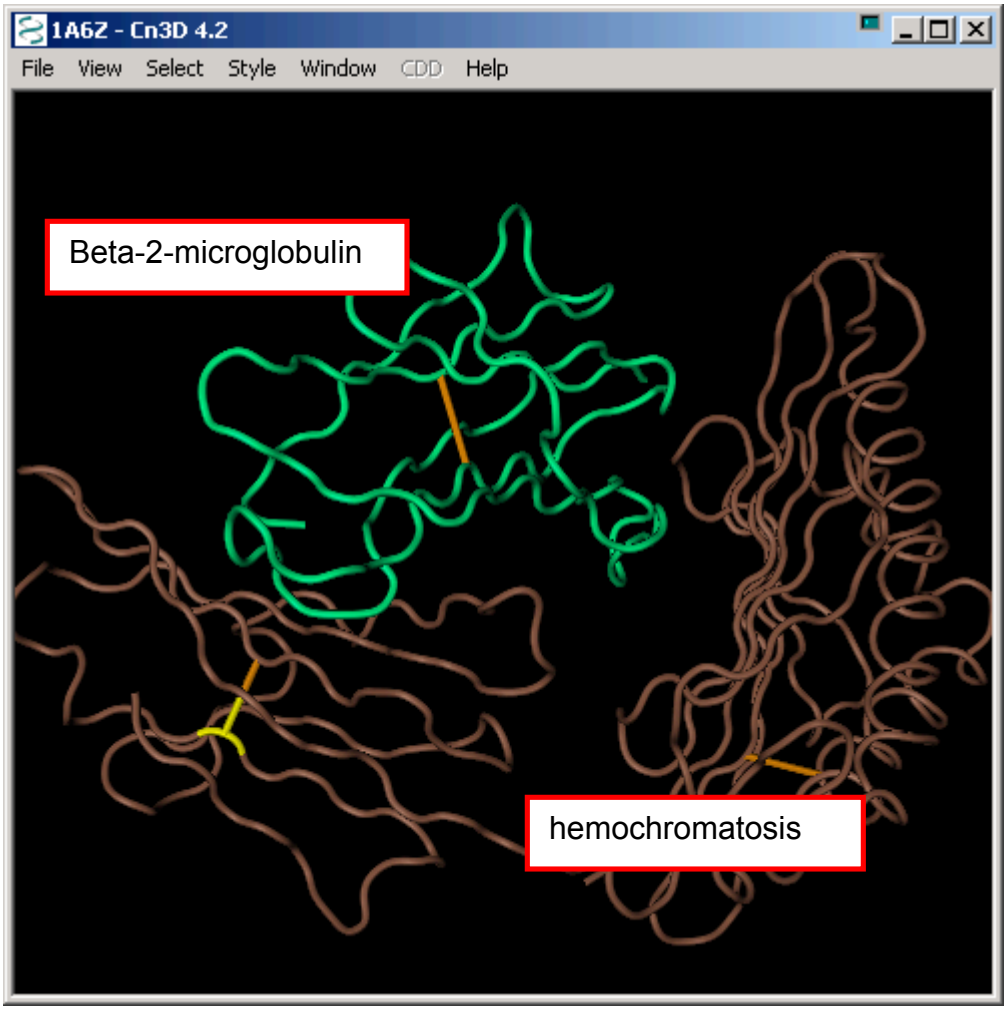
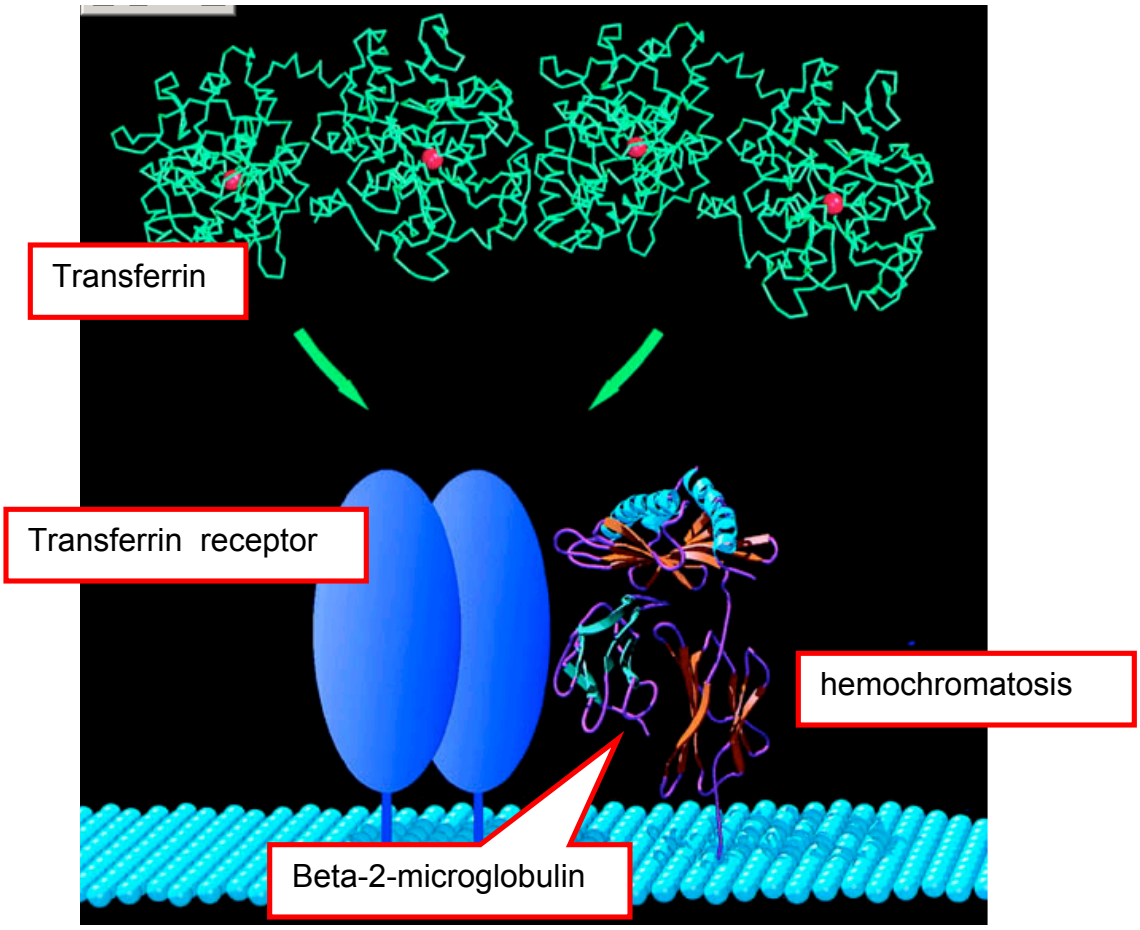
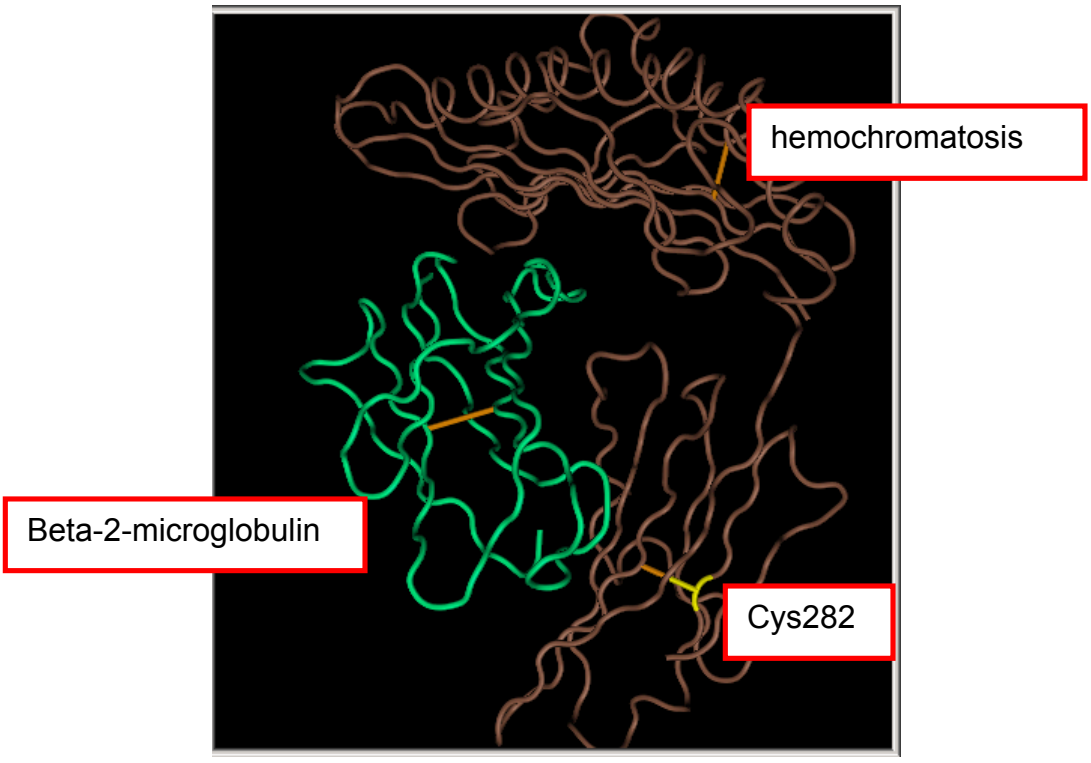




**The interaction of hemochromatosis protein with beta-2-microglobulin allows cell surface presentation of the complex. Once on cell surface, the hemochromatosis protein regulates iron absorption by regulating the interaction of the transferrin receptor with transferrin.**


Transferrin
Transferrin receptor
hemochromatosis
Beta-2-microglobulin


hemochromatosis
Beta-2-microglobulin
Cys282

## Problem 2:

http://www.ncbi.nlm.nih.gov/Class/minicourses/pheno2.html

Mutations in the HBB gene are associated with sickle cell anemia. A laboratory working on sickle cell anemia wants to elucidate the biochemical and structural basis for the function of the mutant HBB protein.

### *Step 1. Determining what is known about the HBB gene and protein (using Entrez Gene):*

Search for 'HBB" in Entrez Gene. One entry is for the human HBB gene. Retrieve the entry by clicking on the HBB link.

What is the location and orientation of the HBB gene on the human genome? List the genes adjacent to it. How many alternatively spliced products have been annotated for the HBB gene when the RefSeq mRNA entries were reviewed? List some of the HBB gene aliases. What are the phenotypes associated with the mutations in the HBB gene?

What is the name and function of the protein encoded by the HBB gene? Beta globin is a subunit of which protein? Name other subunit(s) in that protein.

### *Step 2. Determining other identified SNPs and their locations in the HBB gene:*

From the Links menu on the top right hand side of the page, click on the "SNP: GeneView" to access a list of the known SNPs (reported in dbSNP). By default, the SNPs in the coding region of a gene are reported. Currently, how many coding SNPs are placed on the beta hemoglobin transcript NM_000518? How many of these have links to OMIM? We will concentrate on the Glu7Val mutant in the following analysis.

### *Step 3. Learning more about sickle cell anemia disease and its genetic testing:*

Go back to the Entrez Gene report. Click on the OMIM link and then HBB link. What are the phenotypes caused by mutations in HBB, the absence of HBB and reduced amounts of HBB? How many allelic variants of the HBB gene have been reported? As mentioned in the OMIM report, the allelic variants are listed for the mature beta hemoglobin protein which lacks an initiator methionine. Hence, the allelic variants in the OMIM report are off by one amino acid compared to the precursor protein in NP_000509. Click on the Allelic Variant “View list” link in the left blue bar to get information about the mutant proteins from patients. Is the Glu6Val variant mentioned in the list? (It is the variant number 0243). Which phenotype does it cause? What is the name of the mutant hemoglobin (hemoglobin S).

Click on the Gene Tests link at top of the page. Identify some of the laboratories performing the clinical testing for sickle cell anemia. Now refer to the Reviews section for Sickle Cell Disease, Mutation analysis is available for which of the HBB alleles? List one explanation for the sickle cell anemia phenotype caused by the Glu7Val mutant beta hemoglobin.

## *Step 4. Elucidating the biochemical and structural basis for the function of the wild type and mutant proteins, if possible:*

**A. Information about the wild type protein**
Go back to the OMIM report by clicking the back button on the web browser. Go to the Gene report through the Links menu. Based on the RefSeq summary and the PubMed articles, describe the biochemical functions of beta hemoglobin and hemoglobin S.

Let us first take a look at the structure of the wild type protein. Click on the NP_000509 protein link and select GENPEPT. Click on “Related Structure” from the Links menu. The output contains a list of similar proteins with 3D structures known. The entry, 1DXT_B, represents the structure of deoxyhemoglobin chain B. Click on the arrow next to 1DXT_B to get the sequence alignment of the query protein to the B chain of 1DXT. To view the 3D structure of deoxyhemoglobin (all chains, 2 alpha and 2 beta), click on the MMDB link. That takes us to the MMDB structure summary page for 1DXT. Access the PDB entry, by clicking on 1DXT. Note that the chains A and C in the structure represent alpha chains, and B and D represent beta chains. Go back to the MMDB summary page. View the deoxyhemoglobin tetramer by clicking on the structure image.

Search for the structure of the mutant (deoxyhemoglobin S) in the structure database, if available. Two entries, 1HBS and 2HBS, are retrieved. Click on the 2HBS link. Then click on the PubMed link from the MMDB and PDB entries (under Reference). The abstracts indicate that the mutated valine residue of the beta chain contacts with another hemoglobin tetramer molecule to form hemoglobin polymers which are building blocks for the sickle cell fiber.

**B. To show the side chains of the mutant residue and view its interaction with another hemoglobin molecule:** Download the structure 2HBS by clicking on the structure image on the MMDB page. For easier viewing, remove the helix and strand objects using Style--Edit global style, and unclick the boxes next to the Helix objects and Strand objects. Highlight valine 6 from the H chain (one of the beta chains). To show the side chains of the residue, use the Structure window--Style--Annotate--new. Give a name to this annotation such as "valine" and then click on Edit Style. Change the protein backbone “Rendering” to "Space Fill", Color Scheme to "charge" or "hydrophobicity". Repeat these steps for the Protein Sidechains row and click the Protein Sidechains on. To show the amino acid number, choose the Labels panel, and change the Protein Backbone spacing to 1. Click on the “Done”, “OK” then “Done” buttons. The valine residue

interacts with a pocket between the two helices on another tetramer. Identify the residues from other molecules within 4 angstroms of the valine, use Show/Hide--Select by distance--other molecules. To unselect the highlighted residues, click on the white portion of the sequence window.

***You can now easily explain why the Glu7Val mutant has an altered function.***

**Summary:**

This mini-course describes how to obtain information about the HBB gene, known SNPs in it, and elucidate the biochemical and structural basis for the function of the wild type and Glu7Val mutant protein.

Summary: 1. The HBB gene is located on chromosome 11 and has no alternatively spliced products annotated.
2. Currently, there are 301 coding SNPs annotated on the protein NP_000509.
3. The Glu7Val mutant is associated with the sickle cell anemia disease and the site of mutation is used in sickle cell anemia genetic testing.
4. The HBB gene encodes beta hemoglobin which is a part of hemoglobin along with alpha hemoglobin. Hemoglobin is a tetramer consisting of 2 beta and 2 alpha chains. Mutation of the 7th negatively charged amino acid, glutamic acid, to hydrophobic valine leads to polymerization of hemoglobin forming a sickle fiber that changes the shape of red blood cells leading to sickle cell anemia.